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ARE THERE LIVING BACTERIA IN STONY METEORITES?

BY CHARLES B. LIPMAN

In discussing, about six years ago, my investigations on living bacteria in rocks,[1] with Professor W. J. Mead of the University of Wisconsin, I received the suggestion to make a search of stony meteorites (aërolites) for possible living microörganisms in them. This suggestion was very interesting, and I decided to act thereon if I could obtain proper specimens for study. It would be too long a story to tell here of the difficulties and delays which I experienced in obtaining an adequate number of usable specimens of stony meteorites. Suffice it to say that during the six-year period in question, I have had the good fortune, through gift and purchase, to obtain several good specimens of aërolites, and I have subjected nearly all of them to study by methods described below. Acknowledgments for gifts of meteorite specimens for the purposes of my study are made below. The specimens were all completely or nearly completely crusted and were all small, weighing from fifty grams or less to several hundred grams.

The results of my search for living microörganisms in meteorites, together with my interpretations of them, are given in the following pages. The reader will note that I am not entering into any apology or justification for my study and for this report thereon. I desire to let my statement of findings and my discussion and conclusions respecting them speak for themselves.

GENERAL EXPERIMENTAL TECHNIQUE

It is at once obvious that in any such crucial experiments as these for the determination as to whether living cells exist in stony meteorites, the item of technique of the investigation is of paramount importance. The arrival at the most desirable technique was a matter of evolution, and a number of meteorite specimens had to be sacrificed more or less in the process. The general idea, however, remained the same throughout, viz., an attempt was made to remove from the surface of the speci-

[1]See the following papers:
Lipman, Charles B. 1928. 'Discovery of Living Microörganisms in Ancient Rocks,' Science, LXVIII, No. 1760, pp. 272–273, Sept. 21.
Lipman, Charles B. 1931. 'Living Microörganisms in Ancient Rocks,' Journal Bacteriology, XXII, 3, September.

men all organisms which might be attached to dust or other adhering substances. This was attempted by first washing the surface of the specimen thoroughly with soap and hot water with the aid of a sterile brush. The specimen was then rinsed in distilled water, dried with a paper towel and placed in a solution of a bactericide. At first, solutions of $HgCl_2$ (concentration of 1 to 1000) were used, and periods of exposure thereto varied in different experiments from one to one and one-half hours. Later, superoxol, a 30% solution of H_2O_2, was used for periods varying generally from three to six hours. The substitution of superoxol for $HgCl_2$ was made because of the suspicion that $HgCl_2$ reacts with some of the constituents of the meteorites and therefore remains in them and possibly poisons the media into which they are transferred later. After the exposure of the specimen to the bactericide for the desired period, it was transferred to 95% alcohol for half a minute to a minute, grasped with sterile tongs and exposed to a large gas flame until the alcohol had all burned away and for a few seconds more. In the early experiments, it was then quickly thrown into a sterile iron mortar and crushed, and the powder distributed with a sterile spoon into several flasks of sterile media. In the latter experiments, however, the specimen was dropped directly from the flaming procedure just described into a wide-mouthed flask containing one of the best adapted media in sterile condition. In such media, the specimen remained for periods varying from two or three weeks to four or five months, and if no growth was evident, the supernatant fluid was plated and poured off, the flask being thoroughly flamed before and after opening, and the specimen dropped into a sterile mortar and crushed as described above. The sterile mortars were prepared and guarded with the greatest care and the technique involved was as described elsewhere[1]. Wherever growth appeared in the small culture flasks of liquid medium into which the meteorite powder from the mortar was introduced, it was studied directly under the microscope and by plating. Practically all of the manipulation involved in these experiments was carried out in an inoculation chamber specially sterilized every time it was used, by many hours of fumigation with formaldehyde vapor and steam. Everything used in the experiments was sterilized by the most drastic means. Glassware and tongs were heated for twenty-four hours or more at 165° C. The mortars were heated at the same temperature for several days. Liquid and solid media were sterilized in the autoclave two or three times before using, each exposure being from one to three hours at 20 pounds steam pressure. Except as described

[1]1931. 'Living Microörganisms in Ancient Rocks,' Journal Bacteriology, XXII, 3, September.

otherwise below, incubation of cultures was at 28° C. in a special incubator room, and all culture flasks during and before incubation were protected against contamination by capping cotton stoppers with filter paper which had been dipped in $HgCl_2$ solution.

The foregoing description gives an idea of the technique employed in general, other information with respect to technique being given below in some detail in connection with each experiment described.

PRELIMINARY EXPERIMENTS

Under the designation of preliminary experiments are grouped here arbitrarily all those carried on with meteorite specimens which were not accorded the most refined methods of manipulation developed during the entire study, since results obtained in these earlier, less thoroughly controlled, experiments are less dependable and therefore require separate and briefer discussion. As in the case of the more complete experiments, the results obtained with each specimen and other data relative thereto will be described separately.

Laboratory Number 235

U. S. National Museum, Washington, No. 656. Weight, 69 grams. Found in Ness County, Kansas, 1898.—Mrs. Comley Ward.

Treatment.—Washed as described above, exposed to $HgCl_2$ solution for 1 hour. Rinsed in sterile distilled water. Placed in alcohol, flamed, dropped into sterile mortar. Crushed. Distributed into flasks of liquid media.

Results.—Growth obtained in three out of five flasks of sea-water peptone and tap-water peptone media. Rods of medium length and thickness. Tendency to form chains. Some much shorter than others. Apparently spore-formers. Also long and thin rods forming spores. Also large *Torula*-like cells.

Laboratory Number 238

U. S. National Museum No. 189. Found in Forest City, Iowa.

Treatment.—Washed, exposed to $HgCl_2$ (1 to 500+HCl) for 1 hour. Rinsed in sterile H_2O, placed in alcohol, flamed, dropped into mortar and crushed. Distributed into four flasks of sea-water peptone medium.

Results.—Growth obtained in all cultures. Limited to long rods of medium thickness and short plump rods. Growth good also in other media than one first tried. All rods spore-forming.

Laboratory Number 239

U. S. National Museum No. 654. Found in Ness County, Kansas.

Treatment.—Same as in No. 238.

Results.—Growth obtained in three out of five culture flasks. Rods of medium length, slender, and also shorter plump rods.

Laboratory Number 262

American Museum of Natural History, New York, No. 246. Found in Forest City, Iowa. Weight, 60.9 grams.

Treatment.—Washed, rinsed in distilled water, placed in superoxol for 2 hours, transferred to alcohol, flamed, and dropped into flask of sterile tap-water-peptone medium. After two weeks, medium still clear. Flask flamed, medium poured off, specimen dropped into a sterile mortar and crushed. Powder distributed into tap-water-peptone and into sea-water-peptone media.

Results.—No growth in sea-water-peptone medium. Two out of four flasks in tap-water-peptone medium give growth of very short rods and coccus forms. No growth in nitrification medium. In sea-water-sulphur autotrophic medium, a giant form developed 12μ long $\times$ 5μ wide.

Laboratory Number 283

Ward's Natural Science Establishment, Rochester. Found in Navajo County, Arizona. Fell July 19, 1912. Weight, 54 grams.

Treatment.—Washed, rinsed, placed in superoxol for 3 hours, then in alcohol, flamed and dropped into large flask of tap-water-peptone medium. After two weeks, medium remained clear and plates made therefrom found negative. Specimen again placed in alcohol and flamed, and then dropped into sterile mortar and crushed. Powder distributed into tap-water-peptone medium.

Results.—No growth in any flask. Transfers to sea-water-peptone also negative throughout. Transfers to starch medium show growth in three out of four cultures, all being rods medium length and width, and in addition one coccus form.

Laboratory Number 285

Ward's Natural Science Establishment. Found 1891, Long Island, Phillips County, Kansas. Weight, 56.5 grams.

Treatment.—Washed, placed in superoxol 3 hours, then in alcohol, flamed and dropped into large flask of tap-water-peptone medium. One week later no evidence of growth, flask flamed, medium poured off, specimen again placed in alcohol and flamed, dropped into sterile mortar and crushed. Powder distributed into four flasks each of tap-water-peptone and sea-water-peptone.

Results.—No growth in sea-water-peptone media. Short rods in two out of four tap-water-peptone cultures. Rods rather thick and contain spores.

Laboratory Number 356

Ward's Natural Science Establishment. Found in 1898, Ness County, Kansas. Weight, 156 grams.

Treatment.—Washed, placed in superoxol for six hours, dipped in alcohol, flamed and preserved in sterile beaker for three weeks. Again treated as just described and then dropped into sterile mortar and crushed. Crushing not successful, specimen being very hard—only a little powder obtained. This was distributed into several different media, but no growth was obtained in any culture. The uncrushed part of the meteorite (nearly all of it) was for a third time treated as described above and crushed, this time successfully. Powder distributed in coal-extract-peptone, soil extract+meteorite powder peptone, and algal medium.

RESULTS.—Short bacilli found in all except one flask of soil extract-meteorite powder-peptone. Rods occurring mostly singly, sometimes in pairs. No growth in algal medium. No growth in sulphur-oxidizing, nitrifying or Bastin's sulphate reducing media.

SECONDARY EXPERIMENTS

With the experience gained from the foregoing experiments, it was possible to plan more complete and more uniform procedures in additional studies on new specimens. Hereinbelow will be found notes on such later experiments. Practically every test made with a meteorite specimen is given here, so that the reader may see as nearly as possible the whole picture of these investigations.

Experiments with the first three specimens in this group proved unfortunate, since it was not possible, in the case of any one of them, to free the surface of the specimen from bacteria even by the very drastic treatment of repeated exposures for six hours each time to superoxol. They were all crushed and studied, however, after being treated as indicated below.

LABORATORY NUMBER 357

Ward's Natural Science Establishment. Found 1912, at Holbrook, Navajo County, Arizona. Weight, 71 grams.

TREATMENT.—Washed as described for specimens in preliminary experiments above. Dried and placed in superoxol for six hours. Dipped in alcohol, flamed and dropped into large flask of sterile 1% peptone coal-extract medium. Placed in incubator. In five days, the medium was found to be turbid and growth found therein. The specimen was removed from the medium, washed and re-treated as before except that three hours only were allowed for the exposure to superoxol. Four days later, the medium was found to be turbid and growth found therein. The specimen was removed from the medium, treated as in the second treatment just described, but was not replaced in new medium as a whole, since it seemed impossible to remove all bacteria from the surface by such treatment. Instead the specimen was dipped in alcohol, kept in a large flame for about 20 seconds, dropped into a sterile mortar, crushed and distributed into the following media: peptone coal extract, and soil extract plus meteorite powder.

RESULTS.—In both media, growth developed showing short rods in the peptone coal-extract medium, and short rods and cocci in the soil extract-meteorite powder medium.

LABORATORY NUMBER 358

Ward's Natural Science Establishment. Found at Holbrook, Navajo County, Arizona. Fell July 19, 1912. Weight, 56 grams.

TREATMENT.—Washed and treated as in Series 356 and 357, and dropped into flask containing 150 c.c. 1% peptone coal extract and incubated at 28° C. In five days, the medium was turbid. Specimen was then removed from turbid medium,

scrubbed again, flamed for about 20 seconds and placed in 200 c.c. of fresh sterile medium of the same kind as before. In four days, the medium was turbid again. The specimen was removed and again treated as before. Again turbidity developed in four days, and the same experience resulted in three additional successive treatments. Attempts to sterilize the surface of the specimen were then discontinued, and as in

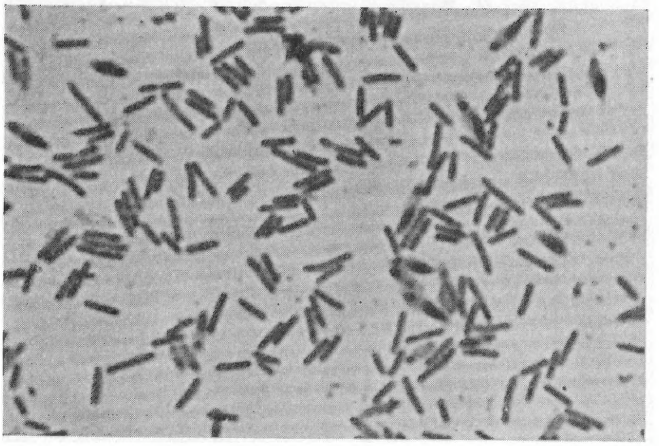

Fig. 1. Isolated from Holbrook Meteorite, Laboratory Series No. 358, 24-hour culture in sodium sulphide peptone soil extract medium. ×1750.

Series 357, the meteorite was thoroughly washed, placed in alcohol and kept in a large flame for 20 seconds. It was then aseptically transferred to a sterile mortar and crushed. The powder was then distributed into sterile peptone coal extract, soil extract plus meteorite powder, and starch medium.

RESULTS.—Of six flasks thus inoculated, two gave growth showing coccus forms and short rods.

LABORATORY NUMBER 359

Ward's Natural Science Establishment. Found May, 1906, Elm Creek, Lyon County, Kansas. Weight, 38 grams.

TREATMENT.—The specimen was treated as in the cases of Series 357 and 358. The same experience resulted in attempts to free the surface of the meteorite of bacteria. After four successive attempts in which the surface of the meteorite yielded growth in sterile peptone coal extract, the specimen was crushed after another treatment for three hours in superoxol, dipping in alcohol and flaming for 20 seconds. The powder was distributed into peptone-coal extract, soil extract plus meteorite powder, and Bristol's Algal Medium. The flasks were then incubated at 28° C.

RESULTS.—Only one flask of the six inoculated with the meteorite powder showed growth. This proved to be bacilli occurring in long chains and a few coccus forms.

GENERAL COMMENTS ON THE FOREGOING EXPERIMENTS AND THE FINAL EXPERIMENTS

The experience gained with the two groups of specimens as detailed above under "Preliminary Experiments" and "Secondary Experiments" leaves one in doubt as to whether bacteria occur in living form in the interior of stony meteorites. However, two important lessons were learned from those experiments. The first lesson was that it is essential to determine beyond question that the surface of the meteorite is free from bacteria before crushing it, and the second lesson was that drastic methods must be employed at the first treatment of a meteorite in order to clear its surface of any living cell. After all the foregoing experiments, therefore, I determined to profit by these lessons and continue the experiments with new specimens and with redoubled vigilance as regards the technique employed, with the results indicated below.

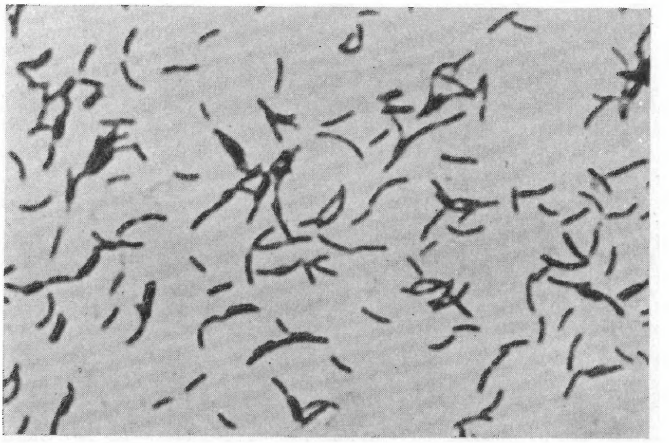

Fig. 2. Isolated from Modoc Meteorite, Laboratory Series No. 377, 24-hour culture in soil extract medium. ×1750.

FINAL EXPERIMENTS

LABORATORY NUMBER 377

American Museum of Natural History, Catalogue No. 2440. Modoc, Scott County, Kansas. Fell 9:30 P.M., September 2, 1905. Veined, white hypersthene-chondrite. Weight, 162 grams.

TREATMENT.—Specimen scrubbed with new soap and hot tap-water, using sterile hand-brush, rinsed in sterile water, and dropped into a beaker of superoxol. Left there for 3½ hours, then removed, rinsed in 95% alcohol, flamed in a large flame and dropped into a flask containing sterile peptone soil extract. This was on March 19.

1931. The medium was still absolutely clear on May 20, 1931. The medium was then quickly poured off, after thoroughly flaming the mouth of the flask, and the specimen was dropped into a sterile mortar and crushed. With a very hot sterile

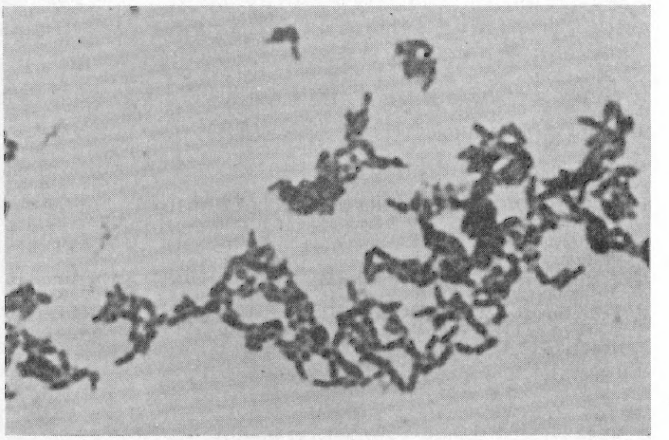

Fig. 3. Isolated from Modoc Meteorite, Laboratory Series No. 377, 24-hour culture in soil extract medium. ×1750.

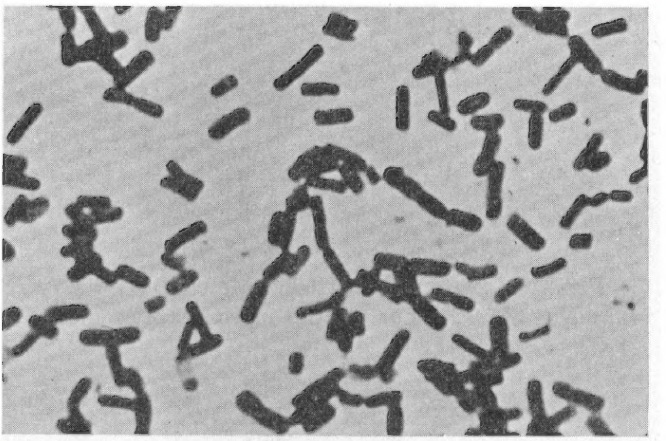

Fig. 4. Isolated from Modoc Meteorite, Laboratory Series No. 377, 24-hour culture in soil extract medium. ×1750.

spoon, the powdered substance was then distributed into several flasks, each of different media as shown below. The solution from the original flask was plated to determine whether it was sterile. No growth except two or three mold colonies developed on these several plates.

RESULTS.—The results obtained with the solution cultures are given in the following table, which summarizes not only examinations of the flask cultures after adequate incubation at 28° C., but also the results of isolation of the organisms concerned after they were plated.

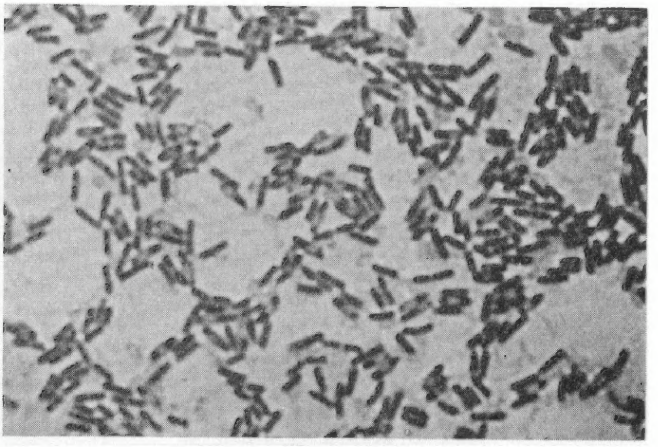

Fig. 5. Isolated from Modoc Meteorite, Laboratory Series No. 377, 48-hour culture in sodium sulphide peptone soil extract medium. ×1750.

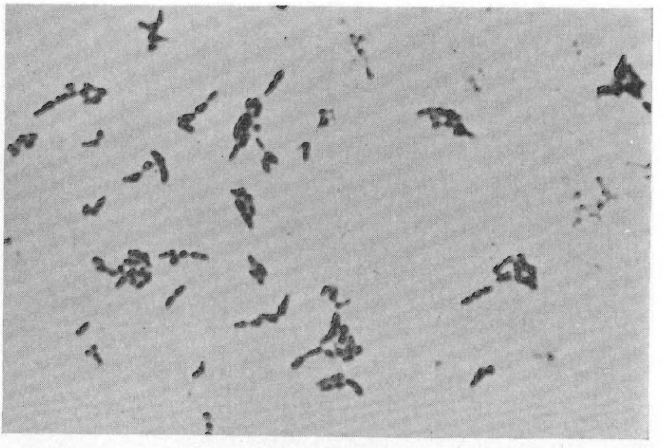

Fig. 6. Isolated from Modoc Meteorite, Laboratory Series No. 377, 24-hour culture in sodium sulphide peptone soil extract medium. ×1750.

TABLE I

Medium	No.	Growth or No Growth	Kinds of Organisms
5% Peptone soil extract	a	+	Rods and coccoid cells.
" " " "	b	+	Medium-sized and minute rods.
" " " "	c	+	Same as Culture *a*.
" " " "	d	+	Rods and coccoid cells.
1% Na_2S peptone soil extract	e	+	Small rods and coccoid forms.
" " " " "	f	+	Same as Culture *e*.
" " " " "	g	+	Same as Cultures *e* and *f* but also larger rods.
" " " " "	h	+	Only a few short rods.
Sodium thiosulphate medium	i	—	———
" " "	j	—	———
" " "	k	—	———
1% Peptone coal extract	l	+	Short rods and coccus forms, but contaminated with a mold.
" " " "	m	—	———
" " " "	n	—	———

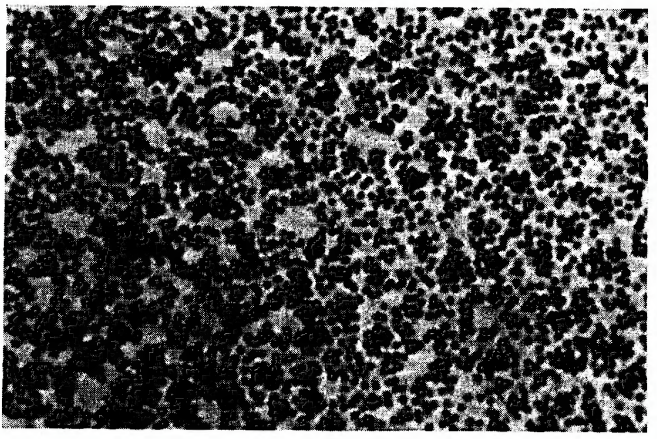

Fig. 7. Isolated from Modoc Meteorite, Laboratory Series No. 377, 24-hour culture in sodium sulphide peptone soil extract medium. ×1750.

Because of lack of space, I am omitting to detail here the description of the many series of dilutions made from these cultures, and of the types of colonies studied on the plates made from each culture flask.

The colonies from the Na_2S peptone soil extract medium yielded mostly coccus forms varying in size, while the peptone soil extract medium yielded mostly rods varying considerably in size. Frequently the cultures showing small rods originally, yielded colonies with medium-sized and even large rods in successive platings. It will be noted that the soil extract peptone and the same medium plus Na_2S were favorable media for the meteorite organisms, but the other two media were apparently

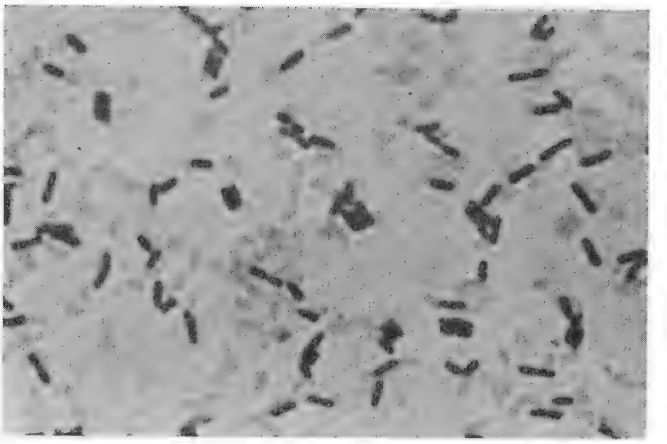

Fig. 8. Isolated from Modoc Meteorite, Laboratory Series No. 377, 4-day-old culture in sodium sulphide peptone soil extract medium. ×1750.

not well suited to their development. Sub-cultures made from the original flasks into still other specialized media gave the following results in summarized form. In Bristol's Algal Medium, incubated in subdued light, coccus and rod forms developed from most of the cultures but not as abundantly as in the more favorable media. In Lieske's sulphur oxidizing medium, only one culture developed a few medium-sized rods. In Jacobsen's sulphur-oxidizing medium, growth was obtained in two transfers from culture *b* (see table I above).

Laboratory Number 388

American Museum of Natural History. Catalogue No. 1155. Holbrook, Navajo County, Arizona. Fell 7:15 P.M., July 19, 1912. Crystalline, spherical, hypersthene-chondrite. Weight, 252.5 grams.

Treatment.—Specimen was scrubbed with hot water and soap, and rinsed in sterile tap- and distilled water, successively. Then placed in a sterile beaker and covered with superoxol. Remained thus for 5 hours with occasional shaking. Re-

moved from superoxol with sterile hot tongs into 95% alcohol. Removed from alcohol with sterile hot tongs to a large flame. After 15 seconds in flame, dropped into a sterile solution of .1% Na_2S–.25% peptone-soil extract. Flask broken by impact of specimen. The latter then again grasped with sterile forceps, dipped in alcohol and flamed for 20 seconds more in a large gas flame and then dropped into another flask of sterile medium like that just described. This was on October 10, 1931. It was incubated at 28° C.

On December 17, 1931, after the medium had remained clear for more than two months, the flask was taken from incubator into a sterile inoculating chamber. Some of the solution was plated to determine sterility. The balance of the solution was then quickly poured off after thorough flaming of mouth of flask, and the meteorite dropped into a sterile mortar (40 hours at 168° to 170° C.) and crushed. With sterile, very hot spoon, the powder was distributed into sterile culture media in Erlenmeyer flasks. The following media were used: Nitrifying medium, nitrogen-free mannite, Bastin's Na_2S peptone soil extract, peptone coal extract, coal extract, Bristol's Algal Medium, Jacobsen's sulphur oxidizing, 2% calcium lactate sea-water, soil extract, and Bavendamm's H_2S medium. All cultures were in duplicate and all incubated as in all other series at 28° C.

RESULTS.—The solution surrounding meteorite which was plated as described above gave no growth whatever.

Growth was obtained in the media inoculated with meteorite powder only in two media, viz., soil extract and Na_2S peptone soil extract. In all these cases, the organisms grew sparsely and were bacilli of medium thickness and length. The bacilli were very variable and in some cases were like egg-shaped cocci.

It is remarkable that growth was obtained at all in these cultures, since, as will be noted above, the meteorite not only received drastic chemical sterilization, but in addition was heated twice in a large flame. The conductive properties of the meteorite are very high because of the large amount of metallic substance therein, and hence some organisms in the specimen must have been destroyed before the stone was crushed.

LABORATORY NUMBER 393

Colorado Museum of Natural History, Denver. Johnstown Meteorite, No. 4450, 123 G. Fell at 4:20 P.M., July 6, 1924.

TREATMENT.—Specimen scrubbed with hot water and soap—sterile brush. Rinsed successively in sterile tap- and distilled water. Immersed dry in superoxol and left there for 4 hours and 25 minutes. Then removed with hot sterile tongs to 95% alcohol. After a few seconds removed from alcohol with hot sterile tongs as before to a large gas flame and heated for about 15 seconds. Then dropped into large flask of sterile medium like that used in Series 388 above. As in all other cases, cotton stopper of flask covered with filter paper cap moistened with $HgCl_2$ solution and tied under mouth of flask. These operations were carried out on January 29, 1932. The flask was then placed in the incubator at 28° C.

On March 10, 1932, the medium having remained clear for about six weeks, the flask was removed from incubator to sterile inoculating chamber, cap and stopper removed and mouth of flask very heavily flamed in large gas flame and solution sur-

rounding meteorite poured off. After this and further flaming of the mouth of the flask, the specimen was dropped into a sterile iron mortar (50 hours at 160° to 165° C.). Specimen was then crushed and distributed into the following media in solution in Erlenmeyer flasks: Jacobsen's sulphur oxidizing, Bavendamm's H_2S medium, Bristol's Algal Medium, Scales' medium minus cellulose, Na_2S peptone soil extract, peptone soil extract, peptone coal extract, coal extract, and Bastin's medium. All cultures were in duplicate, and all incubated as in all other series at 28° C.

RESULTS.—Growth developed quickly in some cultures and slowly in some other cultures. Observations are given in the following table:

TABLE II

Medium	Culture No.	Growth or No Growth	Kinds of Organisms
Peptone coal extract	1	+	Principally large rods (*B. megatherium*) and also small slender rods.
" " "	2	+	Principally large rods (*B. megatherium*) and also small slender rods.
N_2S peptone soil extract	1	+	Small coccus and some small rods.
" " " "	2	+	Small coccus and some small rods.
Peptone soil extract	1	+	Numerous small cocci and some large and small rods.
" " "	2	+	Same rods but no coccus forms.
Jacobsen's sulphur oxidizing	1	+	Medium to large coccus and diplococcus.
	2	—	
Coal extract	1	—	
" "	2	—	
Bastin's	1	+	Large rods (*B. megatherium*), also some slender rods.
"	2	+	Large rods (*B. megatherium*), also some slender rods.
Scales' minus cellulose	1 and 2	—	
Bavendamm's H_2S	1 and 2	—	
Bristol's Algal	1 and 2	—	

LABORATORY NUMBER 394

Colorado Museum of Natural History. Fragment of Johnstown Meteorite. Same source as Series 393 above. Museum Catalogue No. 4443, 49G.

TREATMENT.—This specimen was a small duplicate of the one in Series 393 above. It was treated in the same way. The results are given below.

RESULTS.—Of the several media tested with this small fragment of the Johnstown meteorite as listed in Series 393, only two media in the incubator yielded growth, viz.: In peptone soil extract, large variable rods were obtained in abundance (*B. megatherium*). Occasional coccus forms visible. In Na_2S peptone soil extract, very short rods or egg-shaped coccus forms, and also large variable rods in small numbers (*B. megatherium*). In addition, the Bristol's Algal Medium was examined and found in this case to contain large rods and large ovoid cells, and some fairly large coccus forms. Some cells there also appeared to be yeast-like or *Torula*-like.

Fig. 9. Isolated from fragment of Johnstown Meteorite, Laboratory Series No. 394, 24-hour culture in sodium sulphide soil extract medium. ×1750.

LABORATORY NUMBER 403

Field Museum of Natural History, Chicago. Mocs, Catalogue No. 1447. Fell February 3, 1882. Weight, 109 grams.

TREATMENT.—Specimen treated like that in Series 393, except that only 3 hours and 10 minutes' exposure were allowed for superoxol, and the heating in the open flame after dipping in alcohol was carried out in a specially devised large gas burner with a flame giving a temperature in excess of 1000° C. for about 15 seconds. This was very drastic heating as compared with that used in earlier series and significant for reasons given above. This was done on May 17, 1932. About three weeks later, on June 6, 1932, the specimen was crushed in a mortar sterilized for 80 hours at 155 to 160° C. Inoculations were carried out as before in a thoroughly sterilized inoculation chamber treated with formaldehyde and steam, and cheese-cloth masks were worn by the operators. The meteorite powder was then inoculated into sterile media, as follows: Na_2S peptone soil extract, peptone soil extract, peptone coal extract, Bastin's, Scales' minus cellulose, and Bristol's Algal Medium.

RESULTS.—Control plates exposed in the inoculation chamber after all inoculations had been made. Four plates exposed by passing through atmosphere of chamber three times each, developed no colonies. One plate exposed for one-half minute in

chamber developed one mold colony and the other similarly exposed developed two mold colonies.

Cultures in Na_2S peptone soil extract yielded a number of colonies on the plates, consisting of slender rods of medium length, short rods and cocci.

Cultures in Bastin's, a few colonies consisting of coccus forms, and in one culture an organism like *B. megatherium.*

Cultures in peptone soil extract yielded a number of colonies consisting of short rods.

Other media yielded no colonies.

After heating at 40° C., the cultures gave no growth except those in peptone soil extract which again yielded several colonies consisting of egg-shaped cocci or very short rods.

Plates placed in ice chest at about 5° C. developed no colonies.

Laboratory Number 404

Field Museum of Natural History, Chicago, No. 291. Pultusk. Fell January 30, 1868. Weight, 117.0 grams.

Treatment.—This specimen was treated exactly like the Mocs meteorite in series just preceding, on May 17, 1932. On June 6, it was crushed by the same

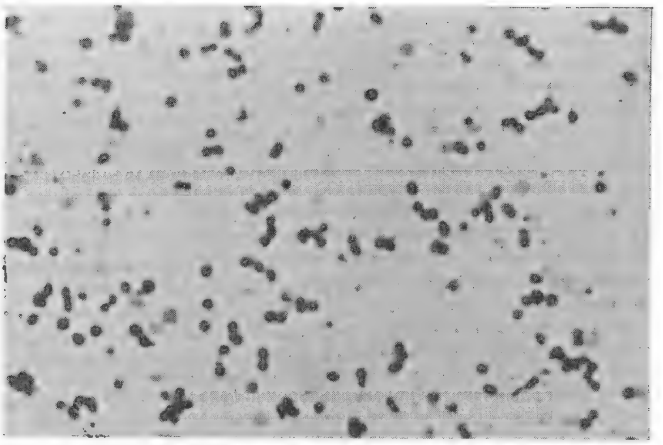

Fig. 10. Isolated from Pultusk Meteorite, Laboratory Series No. 404, 15-day-old culture in peptone coal extract medium. ×1750.

technique as that used in Series 403 and the powder distributed into same kind of media.

Results.—Plates poured with solution of Na_2S soil extract, in which specimen as a whole was incubated, were entirely without growth after several days, and the sterility of the inoculation chamber was tested by the same control plates described above. The plates made after several days' incubation of the original solution cultures made with the meteorite powder gave the following results:

Peptone coal extract: Heavy growth. Very irregularly shaped colonies, small and opaque. Streaked plates gave two types of colonies, one tiny pink and the other larger pink. Both colonies gave the same type of organism, an irregular rod mixed

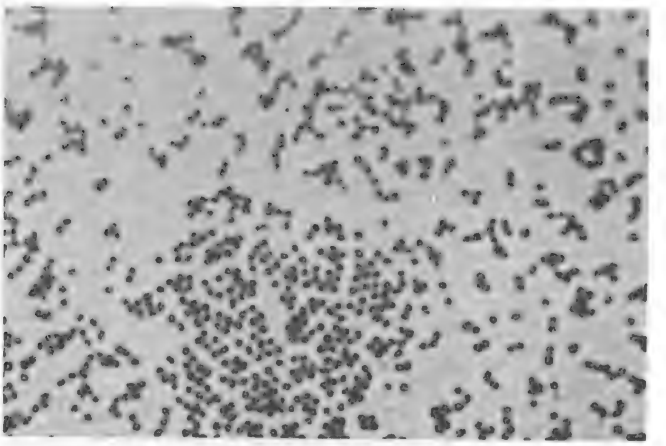

Fig. 11. Isolated from Pultusk Meteorite, Laboratory Series No. 404, 25-day-old culture in Scales' medium minus cellulose. ×1750.

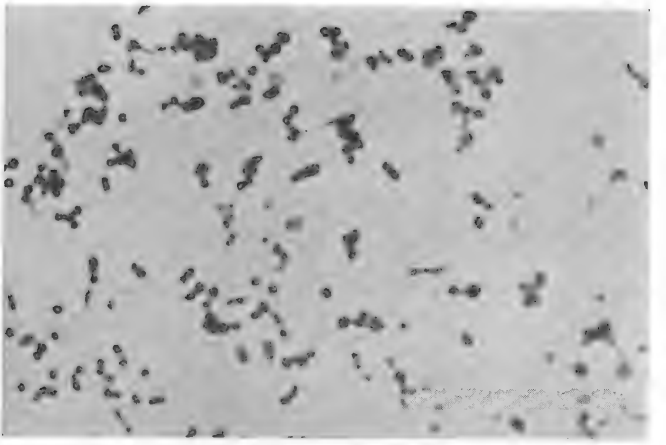

Fig. 12. Isolated from Pultusk Meteorite, Laboratory Series No. 404, 25-day culture in peptone coal extract agar, kept in ice chest at 5° Centigrade. ×1750.

with coccus forms. This is found to be a remarkable organism not only as regards its morphology but also as regards its physiology. It grows well in an autotrophic Scales' medium minus cellulose, rendering the solution turbid, also in peptone media and in Jacobsen's sulphur oxidizing medium (also autotrophic). In Scales' medium

plus paraffine, it attacks the paraffine readily. In the original culture, it started as a peculiar rod in clumps. By sub-culturing, it gradually became transformed into a perfect coccus. At 5° C., it produced polygonal-shaped colonies of two types which again gives a picture of variable rods in branching order along with coccus forms.

Peptone soil extraçt: Only two colonies produced. One consists of a coccus, and one of a large rod. These colonies may be invaders.

Na_2S peptone soil extraçt: Only two colonies produced. One yields an egg-shaped coccus of variable size and shape, and the other a rod of variable size and shape. These colonies may be invaders.

All other media yielded no growth.

At 40° C., no growth was obtained in any of the cultures. The only definite evidence of bacteria in this meteorite was that in one peptone coal-extract culture which yielded the very unusual organism described above in abundance, and this could not have come from any other source than the meteorite itself, considering the conditions of the experiment as described above and the highly unusual nature of the organism. This is especially remarkable since the Pultusk meteorite specimen has lain fallow since 1868.

GENERAL DISCUSSION OF THE STUDY

The evidence submitted in the foregoing pages leads the author to conclude that stony meteorites (aërolites) bring down with them from somewhere in space a few surviving bacteria, probably in spore form but not necessarily so, which can in many cases be made to grow on bacteriological media in the laboratory. These bacteria are similar to forms common on our earth and probably identical with some of our forms. Some of these are pictured in microphotographs which accompany this paper. I realize, of course, that such experiments as I have described above and the conclusion to which I have directed the reader's attention above will be challenged by competent critics, and probably more so by critics who are not competent. Naturally, I do not desire my conclusion to be accepted unless the force of fact and logic are on my side. To assist in clear and critical thinking upon this subject, I submit the following reasons against and for my conclusion and leave the rest to competent judges.

As opposed to the author's conclusion, the following may be urged:

1. Stony meteorites contain very little organic matter for the support of saprophytes.

2. The number of bacteria found per gram of meteorite is evidently very small; hence they may be invaders.

3. The heating of the meteorite in its descent through our atmosphere would destroy bacteria.

4. Some batches of powder from one and the same meteorite yield growth while others do not.

5. While it was lying on the earth, and before being found, water with bacteria may have seeped into the meteorite.

6. Organisms found in these studies are too much like or identical with earth bacteria.

As favoring the author's conclusions, the following answers to the foregoing criticisms should be observed:

1. Stony meteorites do not contain much organic matter, but they do contain some, as is shown in analyses which have been published in meteorite catalogues in respect to organic carbon. In addition, I am publishing in American Museum Novitates No. 589, concurrently with this paper, some data on nitrogen content of stony meteorites which show them all to contain a little combined nitrogen, probably organic in nature. So far as I am aware, these are the only figures known for nitrogen in meteorites.

2. Small numbers of bacteria in meteorites as well as in rocks do not by any means justify the objection above. I have shown elsewhere, and shall soon publish other data to the same effect, that in rocks and rocky matter only a few of many original organisms survive, probably in some resting stage, and that they may exist in the rock here and there sporadically. This is more natural than that they should remain numerous and uniformly distributed in such matter as rocks.

3. Geologists generally have advised me that meteorites do not have an opportunity to become heated internally while traveling through our atmosphere. They burn externally but remain cold internally.

4. The answer to criticism 4 is given under two above.

5. Some specimens studied, notably the Johnstown meteorite, had little or no contact with the earth, being picked up immediately after falling, and hence this argument is invalid.

6. There is no valid reason for believing that bacteria similar to ours on earth might not have been evolved on other planets or in other systems in space.

To all the foregoing, I should like to add that a study of the data and observations given above will render invalid the obvious objection of contaminating or invading organisms as an explanation of my results. Too many cases of growth from inoculated meteorite powder into media are cited. In any given meteorite, like that yielding Series 377 or that yielding Series 393, there are altogether too many positive results to be accounted for by contamination, in the light of the extreme care with which the experimental work was done as described above. In fact, the author is convinced that in his zeal to prevent contamination of his

cultures, he employed measures for sterilization in various forms and phases of the work which were so drastic as to have destroyed some bacteria which were in the meteorites. While occasional organisms observed in the work may well be invaders, most of those described above cannot have been. This is especially true of such rare organisms as the autotrophic form found in Series 393 and the other more remarkable one found in Series 404.

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